sacrificing throughput. FRET was highly sensitive to both known activators and inhibitors of SERCA. We screened a small (1280-compound) chemical library and identified nine compounds that significantly affect 2-color SERCA FLT. Three of these compounds affected FRET in a dose-dependent manner, and all three were found to inhibit SERCA function. Two of the hits were known SERCA inhibitors and the third was novel (Gruber et al., J. Biol. Screen, in press). This assay is being extended to several human isoforms of SERCA, for therapeutic applications to heart failure, muscular dystrophy, diabetes, and cancer. This assay is ready for a large-scale HTS campaign, and is adaptable to numerous protein targets. Spectroscopy was performed in the Biophysical Spectroscopy Center at the University of Minnesota, with assistance from Fluorescence Innovations, Inc. (Greg Gillispie, President). This work was funded by NIH grants to DDT (R01 GM27906, P30 AR0507220), to SJG (AHA 13PRE13230005), and to SLR (R01 HL106189).

2164-Plat
Molecular Basis for Sodium Versus Calcium Binding in the Sodium-Calcium Exchanger
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Na^+/Ca^{2+} exchangers (NCX) and potassium-dependent Na^+/Ca^{2+} exchangers (NCKX) are two related families of transporters involved in Ca^{2+} signaling that function by extruding cytosolic Ca^{2+} (and K^+ in NCKX) in exchange for extracellular Na^+. Previous studies have established that this exchange process is electrogenic and with a $3Na^+$:$1Ca^{2+}$ stoichiometry, and have identified specific acidic amino acids believed to be crucial for ion binding and translocation. Recently the crystal structure of the NCX from *Methanococcus jannaschii* was determined at 1.9 Å resolution, revealing an intriguing transmembrane topology consisting of inverted structural repeats, and the presence of four putative ion binding sites formed by highly conserved residues. Notwithstanding these groundbreaking insights, based on the structure alone several ion occupancy states can be hypothesized that would be compatible with the experimental exchange stoichiometry. Here, we use extensive molecular simulations, free energy calculations and mutational analysis to investigate the occupancy and specificity of the ion binding sites in NCX_Mj. The results permit us to identify a $3Na^+$ bound configuration that is both compatible with the X-ray structure and ranked as the most probable from a thermodynamic standpoint. In addition, two putative Ca^{2+} bound configurations are identified that reconcile anomalous scattering data due to Ca^{2+} with an alternative X-ray structure with Cd^{2+} bound. On the basis of these ion configurations, we investigate the mechanism of alternating-access in this exchanger, and explore the molecular events that trigger the conformational transition between inward- and outward-facing states.

2165-Plat
Structure of Trimeric Calcium/Proton Antiporter Protein YfkE Reveals the Mechanisms of Calcium Efflux and its pH Regulation
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Ca^{2+} efflux by Ca^{2+} cation antiporter (CaCA) proteins is important to maintain Ca^{2+} homeostasis across the cell membrane in all kingdoms of life. CaCA proteins extrude Ca^{2+} out of the cells by utilizing a counter electrochemical gradient of other cations such as Na^+ or H^+ as energy. To understand Ca^{2+}/cation exchange mechanism, we determined crystal structure of the Ca^{2+}/H^+ antiporter protein YfkE from *Bacillus subtilis* at 3.1 Å resolution. YfkE forms a homotrimer confirmed by disulfide crosslinking, and each protomer contains 11 transmembrane helices. The protonated state of YfkE is locked in an inward-facing conformation with a large hydrophilic cavity in each protomer opening to the cytoplasm and ending in the middle of the membrane at the Ca^{2+} binding site, whereas a hydrophobic "seal" closes its periplasmic exit. Two highly conserved helices, TMs 2 & 7, kink toward each other, forming a X-like Ca^{2+} translocation pathway, which is blocked by a histidine residue at the Ca^{2+} binding site. Our structural and functional analyses suggest that Ca^{2+}/H^+ alternating access on each side of the membrane induces the rotation of the kink angles of TMs 2 and 7 and the transition between inward- and outward-facing conformations is mediated by large conformational changes of TMs 1 and 6. These studies not only establish structural bases for the novel mechanisms for Ca^{2+} efflux and its pH regulation, but also shed light on the evolutionary adaptation of different energy modes, Na^+ vs. H^+, in the CaCA protein family.

2166-Plat
Functional Reconstitution of the Mitochondrial Ca^{2+}/H^+ Antiporter Letm1
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The Letm1 (leucine zipper-, EF hand-containing transmembrane protein 1) gene encodes a mitochondrial inner membrane protein, whose depletion severely perturbs mitochondrial Ca^{2+} and K^+ homeostasis. Here we expressed, purified, and reconstituted human Letm1 protein in liposomes. Using Ca^{2+} fluorophore and $45Ca^{2+}$ based assays, we demonstrate directly that Letm1 is a Ca^{2+} transporter, with apparent affinities of cations in the sequence of $Ca^{2+} \approx Mn^{2+} > Gd^{3+} \approx La^{3+} > Sr^{2+} >> Ba^{2+}, Mg^{2+}, K^+, Na^+$. Kinetic analysis yields a Letm1 turnover rate of 2 Ca^{2+}/s and Km of ~25 μM. Further experiments show that Letm1 mediates electroneutral 1 Ca^{2+}/2 H^+ antiport. Letm1 is insensitive to ruthenium red (RR), an inhibitor of the mitochondrial calcium uniporter (MCU), and CGP-37157, an inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger. Functional properties of Letm1 described here are remarkably similar to those of the H^+-dependent Ca^{2+} transport mechanism identified in intact mitochondria.

2167-Plat
Folding and Association of Human Uncoupling Protein-1 in Biological Membranes: Evidence for Multimeric Functional Forms
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Human uncoupling protein-1 (UCP1) is highly expressed in the inner mitochondrial membrane of brown adipose tissue (BAT). The physiologically important proton transport function of UCP1 is tightly linked to its vital thermogenic role, which has been shown to reduce obesity and improve insulin sensitivity. Nevertheless, the structure and mechanism of ion transport for UCP1 are not fully understood. In this study, using the auto-induction method, we have successfully expressed UCP1 in E. coli membranes in high yield. Folding and ion transport of UCP1, extracted from bacterial membranes, were studied after its reconstitution into lipid bilayers. The self-assembly of UCP1 into tetramers in lipid membranes was verified for the first time by circular dichroism and fluorescence spectroscopy, and semi-native gel electrophoresis. UCP1 tetramers in different phospholipid vesicles exhibited highly helical structures, as well as proton transport that was activated by fatty acids and inhibited by purine nucleotides. In addition, the mitochondrial lipid cardiolipin modulated both folding and ion transport function of UCP1 tetramers. Overall, the existence of functional oligomeric forms of UCP1 in the lipid membranes, found in this study, provides important implications for understanding the structure and proton transport mechanism of this protein in BAT, as well as structure-function relationships of other mammalian UCPs in other tissues. Common structural and functional features of human UCPs were explored in our previous studies(1).
1. Hoang, T., Smith, M. D., and Jelokhani-Niaraki, M. 2012. Toward understanding the mechanism of ion transport activity of neuronal uncoupling proteins UCP2, UCP4, and UCP5. Biochemistry 51: 4004-4014.

Platform: Protein-Nucleic Acid Interactions II

2168-Plat
Probing Physical Properties of a DNA-Protein Complex Using Nanofluidic Channels
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Nanofluidic channels have become an important tool to investigate single DNA molecules both from a fundamental polymer physics perspective as well as in e.g. optical mapping techniques. However, less effort has been made to study DNA-protein complexes. A main reason is that the extreme surface-to-volume ratio in the nanochannels causes most proteins to stick to the channel walls. We have recently overcome this problem by coating the channels with a lipid bilayer, thereby eliminating sticking.
RecA is an evolutionary conserved protein involved in homologous recombination and DNA repair, for which it forms helical filaments on single-stranded (ss) DNA in presence of ATP. In vitro RecA-DNA filaments can be formed